Enzymatic Assay of ISOCITRIC DEHYDROGENASE (NADP)
(EC 1.1.1.42)

PRINCIPLE:

DL-Isocitrate + β-NADP $<\xrightarrow{ICDH}>$ α-Ketoglutarate + CO_2 + β-NADPH

Abbreviations:
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
ICDH = Isocitric Dehydrogenase (NADP)

CONDITIONS: T = 37°C, pH = 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycylglycine Buffer, pH 7.4 at 37°C
(Prepare 50 ml in deionized water using Gly-Gly, Free Base, Sigma Prod. No. G-1002. Adjust to pH 7.4 at 37°C with 1 M NaOH.)

B. 6.6 mM DL-Isocitric Acid Solution
(Prepare 5 ml in Reagent A using DL-Isocitric Acid, Trisodium Salt, Sigma Prod. No. I-1252.)

C. 20 mM β-Nicotinamide Adenine Dinucleotide Phosphate Solution (β-NADP)
(Prepare 2 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)

D. 18 mM Manganese Chloride Solution
(Prepare 5 ml in deionized water using Manganese Chloride, Tetrahydrate, Sigma Prod. No. M-3634.)

E. Isocitric Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Isocitric Dehydrogenase (NADP) in cold Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Deionized Water	1.95	1.95
Reagent A (Buffer)	0.50	0.60
Reagent B (DL-Isocitric Acid)	0.20	0.20
Reagent C (β-NADP)	0.15	0.15
Reagent D (Manganese Chloride)	0.10	0.10

Mix by inversion and equilibrate at 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

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UNIT DEFINITION:

One unit will convert 1.0 μmole of isocitrate to α-ketoglutarate per minute at pH 7.4 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 67 mM glycylglycine, 0.44 mM DL-isocitric acid, 1.0 mM β-nicotinamide adenine dinucleotide phosphate 0.60 mM manganese chloride, and 0.03 - 0.06 unit isocitric dehydrogenase (NADP).

REFERENCE:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Vol. 2, 624-627

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for information purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.